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Research Article

ESTIMATION OF ANTIBIOTICS AND SYNTHETIC CHEMICALS TOWARDS BACTERIAL WILT OF POTATO CAUSED BY *RALSTONIA SOLANACEARUM*

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Abstract

Bacterial wilt of potato caused by Ralstonia solanacearum is most severe bacterial disease effecting potato production worldwide.In present research, the effect of different chemicals [Score(24.51%), Topsin-M (70%), Cabrio (60%), Fossi l(29%), Milvet (80%), Forum Top (53%), Excel(80%), Evcin (80%), Electus Super (30%) and Copper Hydroxide (52.4%) and antibiotics [Quinosel (10%), Novamox -LA (15%), Neflox(30%), Gentam(20%), Velocef (29%), Rithmo (44%), Cefcom (52.4%), Cefstar(52.41%) were evaluated against R. solanacearum under in-vitro conditions through inhibition zone technique and then, the combined effect of most effective chemical Score and antibiotic Quinosel was evaluated in-vivo conditions. The findings of the current study suggest that certain chemicals and antibiotics may be considered as an effective tool for the management of R. solanacearum in potato cropping system.

Keywords: Inhibition, disease incidence, potato crop, Score, Bacterial wilt, Quinosel.

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1. INTRODUCTION

Potato is affected by many biotic and abiotic stresses. Considering biotic stress, bacterial wilt of potato that **is** commonly known as brown rot is a destructive and widespread disease caused by the anaerobic, gram-negative bacteria, *Ralstonia solanacearum*, which affects potatoes worldwide in temperate, subtropical, and tropical climates. It was first reported in South Africa in 1914 initially in potatoes and thereafter in several other crops such as tomatoes, peppers, eggplants, peanuts and tobacco (Nortjé, 2015). Having 200 plant species as hosts, this pathogen is responsible for huge yield losses in potato crop (Muthoni *et al.*, 2020). Because of the favorable weather conditions in important potato growing zones of Pakistan, bacterial wilt is expected to cause up to 30% yield losses (Majeed and Muhammad, 2018). The prevalence of bacterial wilt in Punjab province ranged from 24 to 60%. Symptoms lead towards stem wilting and thus towards yellowing of tuber, rottening, brown discoloration of vascular ring and milky ooze from cut tuber. typical symptoms of disease are wilting, yellowing and

stunting of infected plant (Kumari and Ranjan, 2020).

As this disease is causing severe crop losses world widely, numerous management strategies and control methods are being adopted at a large scale. Chemicals show quick response against disease within 2 to 3days after application in epidemic conditions and most probably are available being less expensive as compared to other control methods. (He *et al.*, 2014) used Calcium carbonate (CaCO3) to check its effect on the survival *of Ralstonia solanacearum* in the laboratory and on the control of bacterial wilt in the field. According to laboratory results soils treated with CaCO3 significantly inhibited the survival of *R. solanacearum* and increased the pH by approximately 1.5 units as compared to control. (Solé *et al.,* 2012) have used methyl bromide, vapam and chloropicrin as a soil fumigant for treatment of bacterial wilt of potato caused by *R. solanacearum* that showed significant results against this pathogen. Use of antibiotics is preferred being more efficient, having long lasting effect. Most effective evaluated antibiotic and phytoextracts were further applied in field for disease management. (Manda *et al.*, 2020) used antibiotics such as tetracycline, penicillin and streptomycin that have been proven much efficient in suppressing *R. solanacearum*. (Singh and Jagtap, 2017) used streptomycin against *R. soalanacearum* under laboratory conditions. Streptomycin 20.05 mm showed strongest effect against pathogen followed by antibiotics Gentamycin 17.5mm, Tetracycline 16.5mm and copper hydroxide+ streptomycin 11.95mm. So, in current study different antibiotics at different concentrations were evaluated.

2. MATERIALS AND METHODS

2.1. Isolation, purification and identification of *R.solanacearum*.

Samples with the specific symptoms were collected from Research Area, Institute of Horticultural sciences, University of Agriculture Faisalabad and brought to Phyto-bacteriology. After washing, diseased samples were cut with scissor into small pieces of 2-3 mm along with some healthy portion and sterilized by dipping in 1% sodium hypochlorite (NaOCl) for 30 seconds followed by three washings of distilled water to remove the remaining volume of disinfectant. Nutrient agar (NA) medium was prepared by using autoclave (RTVL-1312, Robus United Kingdom) at 121°C and 15 Psi for 15 minutes and used for growth of bacterial culture. Three pieces of sterilized samples were placed on each plate containing solidified media with the help of inoculating needle sterilized at red hot flame and plates were wrapped with paraffin tape. After labeling, samples containing plates were kept in incubator at 30°C. The bacterial growth was observed after 24 hours. Bacteria was purified by taking from the original plate with the help of sterilized cotton swab and gently streaked on another media containing plate in a zigzag pattern incubated at 30°C for 24 hours. Identification was done through biochemical test by using gram staining technique. Bacterial suspension was prepared on slide by mixing pure culture of *Ralstonia solanacearum* in drop of sterilized water. Suspension was stained and leaved for 30 seconds and washed with distilled water followed by ethanol. One drop of safranin as counter staining was added and washed with distilled water. Slide was observed under microscope and bacteria was observed on the basis of colony colour and with fluidal, irregular white colony colour and appearance (Azeem *et al.*, 2020).

2.2. Pathogenicity test:

Inoculum was prepared from the culture of isolated pathogen by mixing it in distilled water and was inoculated through needle size of 21 gauge in leaves of healthy potato plants. One leaf of potato plant was kept as control by injecting distilled water

and it was observed on daily basis. After occurrence of infection on inoculated leaves, symptomatic portions were collected and further processed for re-isolation and identification of pathogen using NA media and incubated at 30°C for 24 hours.

2.3. Evaluation of Antibiotics and chemicals against R. solanacearum in-vitro conditions

Ten antibiotics such as Novamox-LA(15%), Quinosel(20%), Cefstar(52.41%), Neflox(23%), Trisulpha(53%), Gentam(20%), Rithmo(44%), Inocef(80%), Cefcom(52.4%) and Velosef(29%) and chemicals including Topsin-M(70%), CabrioTop(60%), Fossil(29%), Forum Top(53%),Score(24.51%), Electus super(30%), Excel(80%), Copper hydroxide(52.4%), Evcin(80%) and Milvet(80%) were selected for evaluation against *Ralstonia solanacearum*.100 mL Stock solution was prepared. The percentage of active ingredients of antibiotics and chemicals was divided by 100 and that quantity of antibiotic or chemical was added into 100 mL distilled water. For concentrations C1, C2 and C3, 100 ppm, 200 ppm and 250 ppm of stock solution were prepared. For this purpose, 1mL,2mL and 2.5mL of stock solution was taken and mixed with 100ml distilled water. Inhibition zone technique was used for evaluation of antibiotics and chemicals against *R.solanacearum*. Fresh bacterial culture was streaked down on nutrient agar containing plates**.** Filter paper discs with diameter of around 1 cm were autoclaved and dipped in the prepared concentrations of antibiotic and chemical solutions separately with the help of sterilized forceps and placed at the middle of the plate. One plate was kept as control using distilled water. For each concentration three replicates and one control were maintained and incubated at 30°C for 2-3 days. The readings were taken after every 24hrs, 48hrs and 72hrs of incubation. Inhibition zone was measured. The most effective antibiotics and chemicals were further tested under field conditions.

2.4. Evaluation of Antibiotics and chemicals against R. solanacearum in-vivo conditions

Soil drenching method was used in the field for inoculum application on plants. The most effective antibiotics (Quinosel) and chemical (score) with concentration of 250ppm were applied against pathogen on two different sets of plants separately in the field and data was recorded at specific intervals after treatment. The combination of most effective antibiotic (Quinosel) and chemical (score) with highest efficacy from the initial trials was further applied on another set of plants in the field to evaluate the combined effect of these two agents against the pathogen. One set of plants was inoculated with simple water and kept as control. The results were recorded at regular intervals and disease incidence was rated by keeping in view disease rating scale.

Table# 1 Rating scale to evaluate potato cultivars towards Ralstonia wilt

Ratings	Disease incidence (%)	Variety response
1	0.00	Immune
2	1-9	Highly resistant
3	10-20	Resistant
4	21-35	Moderate resistant
5	36-50	Moderate susceptible
6	51-65	Susceptible
7	66-100	Highly susceptible

(Jahanzaib *et al*., 2017)

3. RESULTS

3.1. Evaluation of different antibiotics against R. solanacearum under lab condition

Maximum inhibition zone was expressed by Quinosel (34.14mm) followed by Novamox- LA (28.46mm), Trisulfa (24.94mm) as shown in (Table 4.2). Analysis of variance Among all treatments, maximum inhibition zone was expressed by Quinosel (34.14mm), followed by Novamox-LA (28.46mm), Trisulpha (24.94mm), Inocef (24.27mm), Neflox(23.77mm), Rithmo (23.64mm), Gentam (21.88mm), Velocef (18.78mm) Cefcom (17.40mm) and Cefstar (15.35mm) as compared to control indicated in (table 2 and fig 1). In case of interaction between treatments and concentrations (T×C) indicated that maximum inhibition zone was expressed at 250ppm and then at 200ppm and 100ppm by Quinosel (37.50, 34.27, 30.66mm) followed by Novamox (32.11, 27.83, 25.44mm), Trisulpha (28.33, 22.55, 21.94mm), Rithmo (26.55, 23.77, 21.00mm), Inocef (27.05, 24.50, 23.27mm), Neflox(27.44, 23.94, 19.55mm), Gentam (23.05, 22.38, 19.27mm), Cefcom(21.51, 18.27, 16.55mm), Velosef (18.44, 15.11, 12.50mm), Cefstar (21.55, 17.38,13.27 mm) as compared to control shown in (table 2 and fig 2). The interaction between treatments and days (T×D) expressed that minimum inhibition zone was developed after 24 hours and the size of inhibition zone increased with time. After 48 and 72 hours the maximum inhibition zone was developed by Quinosel(32.27, 34.44, 37.50mm), Novamox(26.88, 28.72, 29.77mm), Trisulfa (24.55, 24.83, 25.44mm), Inocef (23.44, 24.50, 28.33mm), Neflox (23.94 , 23.27 , 24.11mm), Rithmo (23.94, 23.27, 24.11mm), Gentam (21.44, 21.38, 21.88mm), Cefcom (17.95, 19.27, 19.11mm), Velosef (17.55, 16.94, 16.94mm) and Cefstar (14.55, 15.50, 16.00mm) as compared to control as shown in (table 2 and fig 3).

Table# 2 Impact of different antibiotics on inhibition zone of *Ralstonia solanacearum* under Lab condition

Treatments	Active ingredients (%)	Inhibition zone(mm)
Quinosel	Enrofloxacin 20%	34.14a
Novamox-LA	Amoxycilllin 15%	28.46 b
Trisulpha	Sulfadiazine 53%	24.94 c
Inocef	Ceftriaxone sodium 80%	24.27 cd
Neflox	Florifenicol 23%	23.77 e
Rithmo	Clarithromycin 44%	23.64 cde
Gentam	Gentamicine 20%	21.57 f
Velosef	Cephradine 29%	18.78 g
Cefcom	Ceftazidime 52.4%	17.40 h
Cefstar	Cefepime 52.41%	15.35 i
Control	Distilled Water	0.00 j

*Mean value in the colomn sharing similar letter not differ significantly as determined by LSD test (P<0.05).

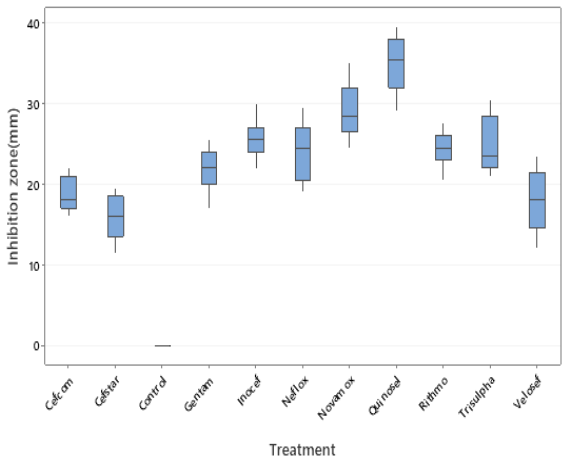

Fig 1 Impact of different antibiotics on inhibition zone against Bacterial wilt of potato caused by *Ralstonia solanacearum* under lab conditions.

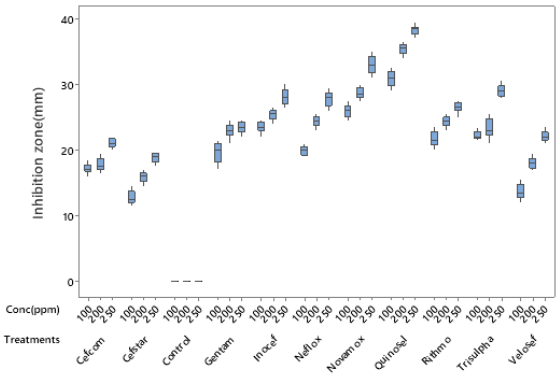

Fig 2 Impact of interaction between treatments and concentrations on development of inhibition zone for *R.solanacearum* under lab conditions.

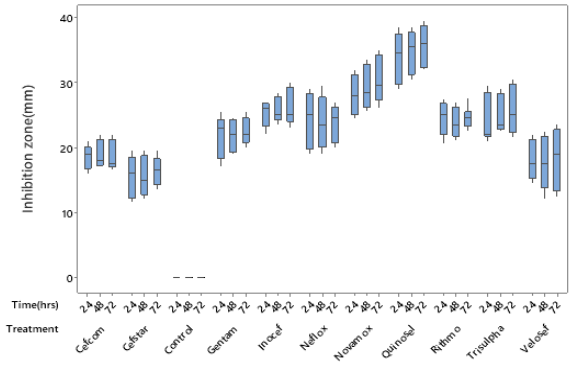

Fig 3 Impact of interaction between treatments and Days on the development of inhibition zone of *Ralstonia solanacearum* under lab condition

3.2. Evaluation of different chemicals against *Ralstonia solanacearum* under lab condition

Experiment showed that maximum inhibition zone was expressed by Score(24.79mm), Fossil(23.46mm), Electus Super (20.25mm), Excel(18.94mm), Forum Top(17.94mm), Cabrio (17.42mm), Copper hydroxide(16.03mm), Evcin(14.48mm), Topsin M(12.42mm), Milvet (8.59mm) as compared to control (table 3 & fig.4). In case of interaction between treatments and concentrations (T×C) indicated that maximum inhibition zone was expressed at 250ppm and then at 200ppm and 100ppm by Score (23.00mm, 24.22mm, 27.16mm) Fossil(20.55,23.72, 26.11mm), Electus Super(19.05, 19.11, 21.05mm), Cabrio(16.66,20.77,20.94mm), Forum Top (17.27, 18.38, 19.61mm), Copper Hydroxide(17.55, 18.33, 19.95mm), Excel(14.22, 15.94, 17.94mm), Evcin(14.05, 14.33, 15.05mm), Topsin M(11.38, 12.05, 13.83mm), Milvet (7.77, 8.66, 9.33mm) as compared to control(table 3 & fig 5). The interaction between treatments and days (T×D) expressed that minimum inhibition zone was developed after 24 hours and the size of inhibition zone increased with time. After 48 and 72 hours the maximum inhibition zone was developed Score(23.00, 24.00, 27.16mm), Fossil (20.56, 23.72, 26.11mm), Electus Super (19.05, 19.11, 21.06mm), Cabrio (16.76, 20.77, 20.94mm), Forum Top (17.27, 18.38, 19.45mm), Copper Hydroxide (17.56, 18.33, 19.52mm), Excel (14.22, 14.94, 17.94mm), Evcin (14.05, 14.33, 15.05mm), Topsin-M (11.38, 12.05, 13.83mm), Milvet (7.77, 8.66, 9.33mm) as compared to control (table 3 & fig 6).

Table# 3 Impact of different chemicals on inhibition zone of *Ralstonia solanacearum* under Lab conditions.

Treatments	**Active ingredients**	**Inhibition zone** (mm)
Score	Difenoconazole24.51%	24.79 a
Fossil	Difenoconazole+ Azoxystrobin29%	23.46 b
Electus super	Difenoconazole+ Azoxystrobin30%	20.25 c
Excel	Azoxystrobin80%	18.94 cd
Forum Top	BAS53%	17.94 cdf
Cabrio	Pyrachlostrobin+ Metiram60%	17.42 dfe
Copper hydroxide	Coside52.4%	16.03 g
Evcin	Cosavet80%	14.48 h

Topsin-M	Thiophenate-methyl70%	12.42 gh
Milvet	Sulfer80%	8.59 hi
Control	Distilled water	0.00 j
LSD	**0.7832**	

*Mean values in the column sharing similar letter not differ significantly as determined by the LSD test (P<0.05)

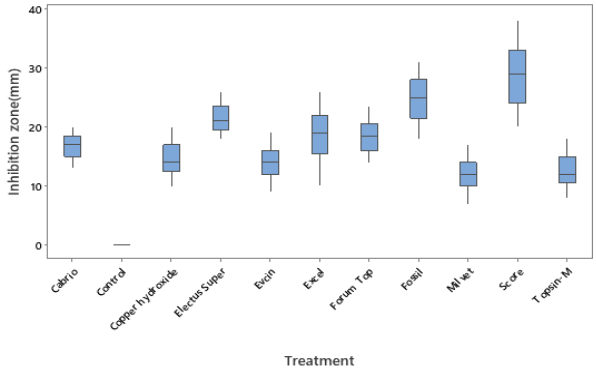

Fig 4 Impact of different chemicals on inhibition zone against bacterial wilt of potato caused by *Ralstonia olanacearum* under Lab conditions.

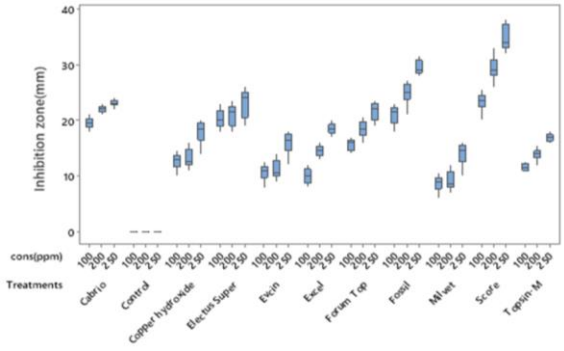

Fig 5 Impact of interaction between treatments and concentration on inhibition zone against Bacterial wilt of potato caused by of *Ralstonia solanacearum* under lab condition.

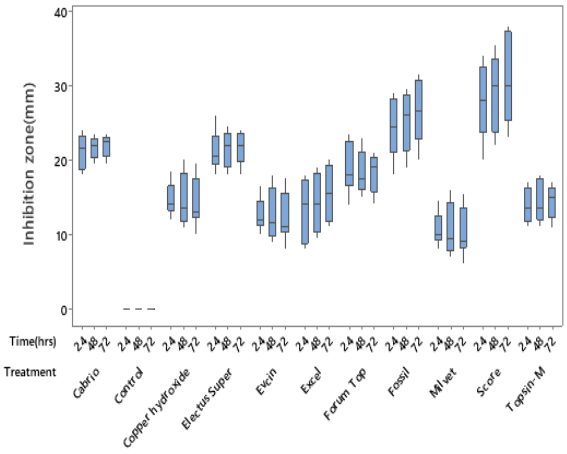

Fig 6 Impact of interaction between treatments and Days on inhibition zone of *Ralstonia solanacearum* under lab condition.

3.3. Evaluation of most antibiotics and chemicals and their combined effect against Ralstonia solanacearum under field condition

Minimum disease incidence was expressed by combination of Score and Quinosel (6.38%) as compared to control (table 4 & fig 7). Interaction between treatments and concentrations (T×C) indicated that minimum disease incidence was expressed at 2.5% and then at2% and 1% by Score (4.00,9.00, 14.00%) and Quinosel (3.11, 8.55, 14.16%) and combination of Score+Quinosel (2.16, 6, 11%) as compared to control (table 4 & fig 8). The interaction between treatments and days (T×D) expressed that maximum disease incidence was recorded after 5 days and disease incidence minimized with time. After 10 and 15 days the minimum Incidence was developed by Score (10.16,9.00,7.83%), Quinosel (9.83,8.66 and 7.33%) and Score+Quinosel (7.66, 6.50, 5.00 %) as compared to control (table 4 & fig 9).

Table# 4 Impact of different antibiotics on Incidence of *Ralstonia solanacearum* under field conditions

Treatments	Active ingredients (%)	Disease Incidence (%)
Score	Difenocnazole 20%	9.00b
Quinosel	Enrofloxacin 15%	8.61c
Score+Quinosel	Difenocnazole 20%+Enrofloxacin 15%	6.38d
Control	Distilled Water	30.01a
LSD	**0.2801**	

* Mean values in the column sharing similar letter are not differ significantly as determined by the LSD test ($P < 0.05$)

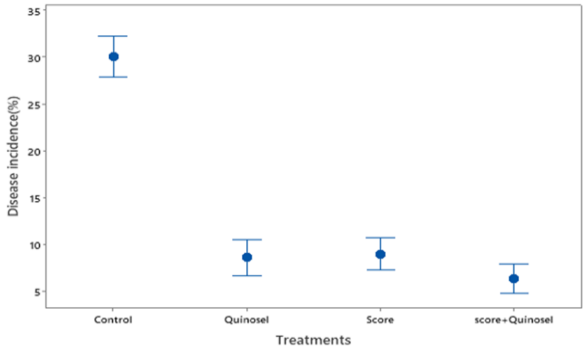

Fig 7 Impact of most effective antibiotic and chemical with their combined effect on Incidence of *Ralstonia solanacearum* under field conditions.

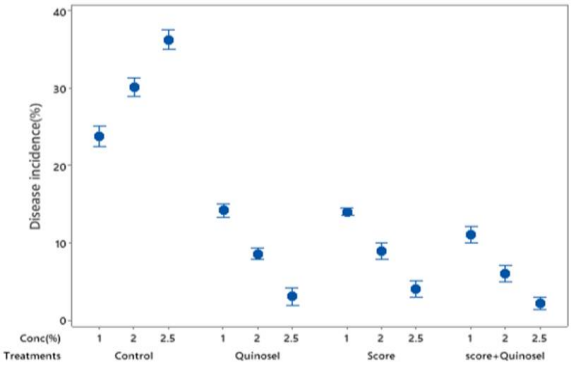

Fig 8 Impact of interaction between treatments and concentrations against *R.solanacearum* under field conditions.

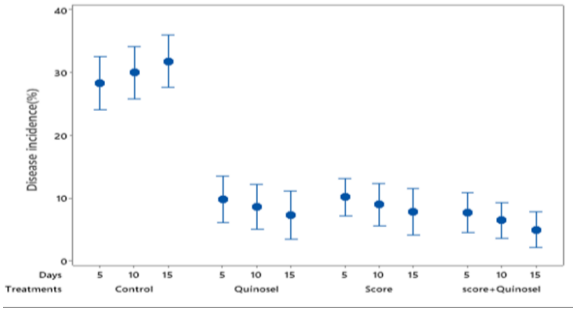

Fig 9 Impact of interaction between treatments and Days on disease Incidence of Bacterial wilt of potato caused by *Ralstonia solanacearum* under field condition.

4. DISCUSSION

Chemicals such as Topsin-M(70%), CabrioTop(60%), Fossil(29%), Forum Top(53%),Score(24.51%), Electus super(30%), Excel(80%), Copper hydroxide(52.4%), Evcin(80%) and Milvet(80%) were selected for evaluation against *Ralstonia solanacearum*.100 mL Stock solution was prepared. The percentage of active ingredients of antibiotics and chemicals was divided by 100 and that quantity of antibiotic or chemical was added into 100 mL distilled water. For concentrations C1, C2 and C3, 100 ppm, 200 ppm and 350 ppm of stock solution were prepared**.** Maximum inhibition zone was expressed by Score (24.79mm), Fossil(23.46mm), Electus Super (20.25mm), Excel (18.94mm), Forum Top (17.94mm), Cabrio (17.42mm), Copper hydroxide (16.03mm), Evcin (14.48mm), Topsin M (12.42mm), Milvet (8.59mm) as compared to control. Score and fossils were evaluated as best chemical against *R.solanacearum* causing bacterial wilt of potato in lab conditions. (Solé *et al.*, 2012) have used methyl bromide, vapam and chloropicrin as a soil fumigant for treatment of bacterial wilt of potato caused by *R.solanacearum* that showed significant results against this pathogen. (Enfinger *et al.*, 1979) have used methyl bromide, vapam, and chloropicrin in the field for treatment of bacterial wilt of potato caused by *R.solanacearum* in which

chloropicrin and methyl bromide were much significant for the control of *R.solanacearum* under optimum conditions, in potatoes.

Ten different antibiotics (Quinosel, Novamox, Rithmo, Trisulfa, velocef, Gentam, Inocef, cefstar, Cefcom, Velocef) under lab conditions were tested against *R.solanacearum* Causing bacterial wilt of potato. Three concentrations were prepared for each antibiotic in ppm (parts per million) from lesser to higher like 100, 200, and 250 ppm. Out of ten antibiotics Quinosel and Novamox were evaluated as the most effective antibiotics against *R.solanacearum* under lab conditions. Persley (1986) founded Streptomycin, tetracycline, erythromycin, nolidixic acid, and kanamycin effective against the *R.solanaearum* under laboratory conditions. Among the tested, antibiotics, Amoxycillin was found to be highly effective with the maximum inhibition range of 31.25 to 36.00 mm at 500 to 750 ppm, Cefixime recorded an inhibition zone of 31.25 to 35.50 mm. Ciprofloxacin recorded as an effective antibiotic having an inhibitory zone of 28.75 to 32.75 mm followed by Tetracycline (24.25 to 27.25 mm), Norfloxacin (20.75 to 22.75 mm) and Streptomycin (14.75 to 16.25 mm). The least inhibitory zone of 7.25 to 10.00 mm was recorded by Bactrimil at 500 and 750 ppm for brinjal (Bawari and Narendrappa, 2019). The combined effect of antibiotics and chemicals (Quinosel+score) was tested on plants in the field. Minimum disease incidence was recorded by (Quinosel+ score) at a concentration of 250 ppm. The same findings were found by (Yuan *et al.*, 2012) in which chemical compounds and extracts were used to control *R.solanacearum* in tomatoes. (Sarkar *et al.,* 2019) found that enrofloxacin (Quinosel) is utilized to control gram-negative bacteria and inhibits class 2 topoisomerases, gyrase, and topoisomerase IV.

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